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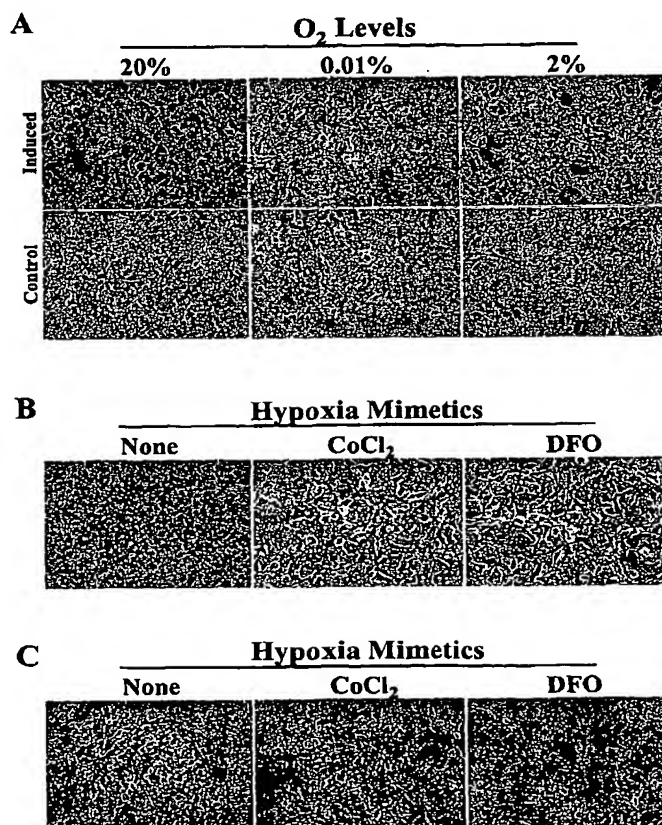
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(54) Title: METHODS AND COMPOSITIONS FOR REGULATING ADIPOGENESIS



(57) Abstract: According to the disclosure hypoxia-mediated adipogenic inhibition involves the repression of *PPARγ2* expression and its activity is a common mechanism for adipogenic inhibition by a variety of stimuli. The present disclosure relates to methods and compositions for regulating adipogenesis. The disclosure provides compositions comprising one or more *DEC1/Stra13* fragments of capable of inhibiting *PPARγ2* promoter activity. These fragments, *e.g.* the basic helix loop helix domain or amino acids 1-141, have substantially the same *PPARγ2* promoter repressing activity as the full length polypeptide. The present disclosure provides methods of inhibiting adipogenesis comprising contacting a cell with a fragment of *DEC1/Stra13*. The invention further relates to methods and compositions of inhibiting angiogenesis in a tumor comprising contacting a tumor or tumor cell with a *DEC1/Stra13* agonist.

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METHODS AND COMPOSITIONS FOR REGULATING ADIPOGENESIS

SPECIFICATION

5 **BACKGROUND OF THE INVENTION**

This application claims the benefit of U.S. Provisional Application No. 60/360,689 filed February 28, 2002.

This invention was made with government support under National Institutes of Health Grants CA88480 and CA67166 and under National Institutes of Health Cancer Biology Training Grant CA09302. The government has certain rights
10 in the invention.

Molecular oxygen (O₂) is vital to nearly all forms of lives on earth perhaps via its role in energy homeostasis, embryogenesis and differentiation. In response to hypoxia or low O₂ tensions, mammals increase the expression of a wide
15 variety of genes including erythropoietin, vascular endothelial growth factor (VEGF) and glycolytic enzymes to stimulate erythropoiesis, angiogenesis, and glycolysis (Bunn and Poyton, 1996). Most of these hypoxia-regulated genes are transcriptionally induced by the hypoxia-inducible factor-1 (HIF-1), a member of the basic helix-loop-helix *Per*, *AhR* and *Sim* (bHLH-PAS) family (Semenza and Wang,
20 1992; Wang et al., 1995a). Under normoxia, HIF-1 α protein becomes hydroxylated at proline-564 in its O₂-dependent degradation domain (Ivan et al., 2001; Jaakkola et al., 2001), and is targeted by the von Hippel-Lindau (VHL) protein for proteasome-mediated degradation (Maxwell et al., 1999; Ohh et al., 2000). Under hypoxia, HIF-1 α becomes stabilized, translocates to the nucleus, and dimerizes with the O₂-
25 independent HIF-1 β to initiate gene expression (Jewell et al., 2001; Kallio et al., 1997). The importance of cellular responses to hypoxia in development and differentiation is demonstrated in mouse models in which homozygous deletion of either *HIF-1 α* or *HIF-1 β* is embryonically lethal. The *HIF-1 α* ^{-/-} embryos succumb between 9 and 10 days post coitus (d.p.c) to loss of mesenchymal cells and impaired
30 vascular development (Iyer et al., 1998; Ryan et al., 1998). The *HIF-1 β* ^{-/-} embryos

die by 10.5 d.p.c due to vascular deficiencies in the yolk sac and/or placenta (Kozak et al., 1997; Maltepe et al., 1997). Interestingly, mice heterozygous for *HIF-1 α* exhibit increased weight loss when subjected to chronic hypoxia (Yu et al., 1999), reinforcing the essential and complex role *HIF-1 α* plays in cellular homeostasis in a low O₂ environment.

During the first trimester, a human embryo is located in a low O₂ environment (3% O₂) (Rodesch et al., 1992). In rat embryos, O₂ tensions are low before 9.5 d.p.c (Mitchell and Yochim, 1968). The establishment of uteroplacental circulation relies on cytotrophoblast invasion into the uterine spiral arterioles. Studies indicate that cytotrophoblasts proliferate with a poorly differentiated phenotype at low O₂ tensions and differentiate into a highly invasive phenotype at high O₂ tensions (Caniggia et al., 2000; Genbacev et al., 1997). High O₂ tensions also favor terminal differentiation of megakaryocytes into platelets (Mostafa et al., 2000). In contrast, differentiation of other cell types seems to prevail at lower O₂ tensions. At 3% O₂, rat mesencephalic precursor cells exhibit higher growth rates and higher levels of differentiation into a dopaminergic phenotype than at 20% O₂ (Studer et al., 2000). Low O₂ tensions have been found to promote osteochondrogenesis. Mesenchymal stem cells from rat bone marrow display enhanced colony-forming capability and increased proliferation at 5% O₂ as compared to 20% O₂, and they produce more osteocytes when implanted *in vivo* (Lennon et al., 2001). These observations suggest that the effect of O₂ on cell differentiation is extensive and cell-type specific.

Peripheral evidence in the literature supports a role of HIF-1 in adipogenesis. Using a subtraction cloning approach, Imagawa et al. (1999) found that *HIF-1 α* mRNA is transiently induced in 3T3-L1 (L1) preadipocytes upon treatment with the adipogenic hormone cocktail containing insulin, dexamethasone and 3-isobutyl-1-methylxanthine (IDM). However, the consequence of such transient *HIF-1 α* expression was never investigated. Interestingly, adipogenesis can be inhibited by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which requires the aryl-hydrocarbon receptor (AhR), also a member of the bHLH-PAS family (Alexander et al., 1998; Phillips et al., 1995). Since AhR activates gene transcription by dimerization with HIF-1 β (Probst et al., 1993), it is a reasonable hypothesis that inhibition of adipogenesis may be a function shared by some members of the bHLH-PAS family such as HIF-1 α/β and AhR/HIF-1 β .

Pathophysiological evidence exists that suggests a correlation between hypoxia and adipogenesis. For example, children with cyanotic heart disease have less body fat due to apparent adipocyte hypocellularity (Baum and Stern, 1977). High altitude training is well known to cause weight loss that is attributed in large part to body fat reduction (Armellini et al., 1997; Westerterp et al., 1994a). Strenuous physical training, on the other hand, is also attributed to significant loss of body fat (Van Etten et al., 1994; Westerterp et al., 1994b). Besides other physiological changes, hypoxia occurs in exercising skeletal muscles, as characterized by an increase in the expression of HIF-1 and VEGF (Gustafsson and Kraus, 2001; Gustafsson et al., 1999). Under hypoxia, fatty acid oxidation is impeded and glycolysis is augmented to maintain energy homeostasis. If the stored fat is not used under hypoxia, there is less need to increase or renew adipose tissue via adipogenesis. Experimentally, rats exposed to hypoxia experience significant fat loss (Mortola and Naso, 1997; Tanaka et al., 1997). Thus, reduction of adipose tissues can be caused by tissue hypoxia.

Adipocyte differentiation results from sequential induction of transcription factors *C/EBP β* , *C/EBP δ* , *PPAR γ* , and *C/EBP α* (Rangwala and Lazar, 2000; Rosen and Spiegelman, 2000). *C/EBP β* and *C/EBP δ* are induced immediately but transiently upon IDM treatment to mediate the expression of *PPAR γ* and *C/EBP α* (Christy et al., 1991; Wu et al., 1995; Yeh et al., 1995). In contrast to *C/EBP δ* , *C/EBP β* is able to induce spontaneous differentiation in L1 cells and enhance the adipogenic potential in NIH-3T3 fibroblasts (Wu et al., 1995; Yeh et al., 1995). Highly specific for adipose tissues, *PPAR γ* plays a critical role in the expression of most adipocyte-specific genes (Tontonoz et al., 1995) and is able to convert non-adipogenic mesenchymal cells such as fibroblasts and myoblasts to adipocytes (Hu et al., 1995; Tontonoz et al., 1994). Although developmentally necessary for adipogenesis (Wang et al., 1995b), *C/EBP α* is not always expressed during adipocyte differentiation especially in cells that already express *C/EBP β* . For example, *C/EBP α* is not involved in the expression of *GLUT-4*, the insulin-responsive glucose transporter, in 3T3 cells ectopically expressing *C/EBP β* and *C/EBP δ* (Wu et al., 1998). These data suggest the *PPAR γ* and *C/EBP β* may be potential targets for adipogenic intervention.

The effects of hypoxia are manifested by HIF-1 regulated genes. We and others have identified a hypoxia-induced gene *DEC1/Stra13*, a member of the *Drosophila hairy/Enhancer of split* (HES) family of bHLH transcription factors (Ivanova et al., 2001). The HES proteins play important roles in cell differentiation by repressing gene expression (Kageyama and Ohtsuka, 1999; Staal et al., 2001). During embryonic development, *DEC1/Stra13* is expressed in neuroectoderm, and in some mesoderm and endoderm derived structures (Boudjelal et al., 1997). In P19 embryonal carcinoma cells, overexpression of *DEC1/Stra13* promotes neuronal differentiation and inhibits mesodermal and endodermal differentiation (Boudjelal et al., 1997). In differentiating L1 cells, *DEC1/Stra13* expression is increased approximately 2-fold within 1 hr of IDM treatment, followed by a rapid decrease within 24 hr (Inuzuka et al., 1999). At present, the role of *DEC1/Stra13* during adipogenesis is ill defined.

Given the importance of O₂ sensing in embryonic development, as well as energy homeostasis and cell differentiation, O₂ tensions may control adipose tissue function by regulating adipogenesis. Since fatty acid metabolism requires mitochondrial respiration, hypoxia prevents the use of fatty acids and thus may obviate the need for more adipose tissue. Therefore, we have investigated whether hypoxia inhibits adipogenesis through the HIF-1 dependent induction of the *DEC1/Stra13* gene expression. As mentioned above, adipocyte differentiation *in vitro* is determined by precisely orchestrated expression of the *C/EBPs* and *PPARγ*. We have thus determined whether the *C/EBP* family or *PPARγ* is the critical target of *DEC1/Stra13*, and whether overexpression of *DEC1/Stra13* is sufficient to inhibit adipocyte differentiation. The regulation of adipogenesis by hypoxia opens new directions for research in understanding how the microenvironment regulates cell differentiation both under physiological settings as well as during the malignant progression of tumors.

SUMMARY OF THE INVENTION

Cellular differentiation involves transcriptional responses to environmental stimuli. Adipocyte differentiation is inhibited under hypoxic conditions, indicating that oxygen (O₂) is an important physiological regulator of adipogenesis. Hypoxia inhibits *PPARγ2* nuclear hormone receptor transcription and

overexpression of *PPAR* γ 2 or *C/EBP* β stimulates adipogenesis under hypoxia. Mouse embryonic fibroblasts deficient in hypoxia-inducible transcription factor-1 α (HIF-1 α) are refractory to hypoxia-mediated inhibition of adipogenesis. The HIF-1 regulated gene *DEC1/Stra13*, a member of the *Drosophila hairy/Enhancer-of-split* transcription repressor family, represses *PPAR* γ 2 promoter activation and functions as an effector of hypoxia-mediated inhibition of adipogenesis. These data indicate that an O₂-sensitive signaling mechanism regulates adipogenesis.

Thus, in some non-limiting embodiments of the present invention, agents that regulate HIF-1 activity or O₂-sensing may be used to inhibit adipogenesis and control obesity. For example, a cell may be contacted with a molecule that inhibits *PPAR* γ 2 transcription and/or activity. These molecules may act by directly inhibiting *PPAR* γ 2 transcription (e.g. the molecule binds to the *PPAR* γ 2 promoter, thereby hindering transcription factor access) or translation (e.g. the molecule is an antisense *PPAR* γ 2 nucleic acid). Alternatively, these molecules may be *PPAR* γ 2 agonists or antagonists. These molecules may also act indirectly by stimulating and/or activating *DEC1/Stra13*.

In some preferred embodiments, the invention provides a *DEC1/Stra13* agonist comprising a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR* γ 2 promoter repressing activity as full-length *DEC1/Stra13* polypeptide. A *DEC1/Stra13* agonist of the invention preferably comprises the basic helix loop helix domain of *DEC1/Stra13*. *DEC1/Stra13* agonist of the invention may have an amino acid sequence selected from the group consisting of amino acids 1-141 of SEQ ID NO:2, amino acids 1-141 of SEQ ID NO:4, and amino acids 1-141 of SEQ ID NO:7. A nonlimiting example of a DEC1 agonist of the invention is a truncated DEC1 polypeptide having substantially the same *PPAR* γ 2 promoter repressing activity as a full-length DEC1 polypeptide, wherein the truncated polypeptide consists essentially of a polypeptide having the amino acid sequence of amino acids 1-141 of SEQ ID NO:2. A nonlimiting example of a Stra13 agonist of the invention is a truncated Stra13 polypeptide having substantially the same *PPAR* γ 2 promoter repressing activity as a full-length Stra13 polypeptide, wherein the truncated polypeptide consists essentially of a polypeptide having the amino acid sequence of amino acids

1-141 of SEQ ID NO:4. The invention further provides isolated nucleic acids encoding a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR γ 2* promoter repressing activity as full-length *DEC1/Stra13* polypeptide.

5 In further non-limiting embodiments of the invention, agents that regulate HIF-1 activity or O₂-sensing may be used to inhibit angiogenesis and treat cancer. These embodiments are based, in part, on the observations that (a) hypoxia and *DEC1/Stra13* overexpression inhibits transcription of *PPAR γ 2*; (b) *PPAR γ 2* is localized in tumor endothelial cells (Inoue K et al., 2001); and (c) *PPAR γ 2* knockout
10 mice display reduced placental vascularization (Barak Y et al., 1999). Thus, without being restricted to any mechanism of action, Applicants hypothesize that hypoxia or agents that target HIF-1 α , *DEC1/Stra13*, and/or *PPAR γ 2* may inhibit angiogenesis and, therefore, may be used to treat cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

15 The patent application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the United States Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1. Hypoxia inhibits adipogenesis

20 (A) L1 cells were induced to differentiate (Induced) or left uninduced (Control) at indicated O₂ tensions. Cells were stained on Day 6 with Oil Red O and photographed (x20).

 (B) L1 cells were induced to differentiate in the presence or absence of CoCl₂ (50 μ M) or DFO (100 μ M) for the first 2 days together with IDM, and then
25 maintained in media without CoCl₂ or DFO. Cells were stained on Day 6 and photographed (x20).

 (C) MEFs were treated in DM containing 5 μ M rosiglitazone with or without CoCl₂ (25 μ M) or DFO (25 μ M). Cells were stained on Day 6 and photographed (x20).

30 Figure 2. HIF-1 is required for hypoxia-mediated inhibition of adipogenesis

MEFs with *HIF-1* α alleles flanked by *loxP* sites were incubated with cre-adenovirus (Cre) or control adenovirus (Control) and induced to differentiate as described. CoCl_2 or DFO was added at the indicated final concentrations for the entire course of treatment. Cells were stained on Day 7 and photographed (x20).

5 Figure 3. Hypoxia modulates the expression of *PPAR* γ 2, *C/EBP* β and *C/EBP* δ

(A) and (B) L1 cells were induced to differentiate either under normoxia or hypoxia (0.01% O_2). Total cellular RNA was prepared at indicated times after induction. Equal amounts (10 $\mu\text{g}/\text{lane}$) of total RNA were subjected to Northern blotting (A) using ^{32}P -labeled *PPAR* γ 2, *C/EBP* β or *C/EBP* δ cDNA as probes. The
10 relative levels of expression were analyzed by densitometry (B).

(C) L1 cells were induced to differentiate under the following conditions: 20% O_2 , 50 μM CoCl_2 , or 100 μM DFO. Total RNA (5 $\mu\text{g}/\text{lane}$) was analyzed as in (A).
15

(D) MEFs were treated as in Figure 2. Total RNA was prepared 3 days after hormonal stimulation with or without 25 μM CoCl_2 or 12.5 μM DFO. Northern blotting analysis (10 $\mu\text{g}/\text{lane}$ RNA) was done as in (A).

Figure 4. Ectopic expression of *C/EBP* β or *PPAR* γ 2 restores the adipogenic potentials of 3T3-L1 cells under hypoxia.

20 (A) Stable *C/EBP* β - or vector-cells (L1) were induced to differentiate (Induced) or left uninduced (Control) either at 20% or 2% O_2 . Cells were stained on Day 6 and photographed (x20).

(B) Stable *C/EBP* β - or vector-cells (L1) were induced to differentiate at 20% O_2 in the presence of either 50 μM CoCl_2 or 100 μM DFO for the first 2 days of
25 induction (2 Days) or the entire 6 days of treatment (6 Days). Cells were stained on Day 6 and photographed (x20).

(C) L1 cells transiently infected with *PPAR* γ 2 or vector control were induced to differentiate under the following conditions, 20% O_2 , 2% O_2 , 50 μM CoCl_2 (6 days), or 100 μM DFO (6 days). Cells were stained on Day 6 and photographed (x20).

30 Figure 5. *DEC1*/*Stra13* expression is regulated by O_2 tensions via HIF-1

(A) Total RNA was prepared from *HIF-1* $\alpha^{-/-}$ or wild-type MEF cells at

indicated time at 0.01% O₂. Equal amounts (10 µg/lane) of total RNA were subjected to Northern blotting analysis with ³²P-labeled *Stra13* cDNA as probe.

(B) NIH-3T3 cell lysates were prepared at indicated time at 0.01% O₂ or during reoxygenation following 24 hr hypoxia. Normoxic controls were prepared at 0 hr and 48 hr, respectively. *DEC1/Stra13* protein was analyzed by Western blotting (25 µg protein/lane) as described.

(C) L1 cells were induced to differentiate either under normoxia or hypoxia (0.01% O₂). Equal amounts (10 µg/lane) of total RNA prepared at indicated time after induction were subjected to Northern blotting as in (A). The relative levels of *DEC1/Stra13* mRNA were analyzed by densitometry (E).

(D) L1 cells were induced to differentiate as in (C). Cell lysates were prepared at indicated times after treatment and subjected to Western blotting (20 µg protein/lane) as described. The controls (lane C) were maintained under either normoxia or hypoxia without adipogenic stimulation. The relative levels of *DEC1/Stra13* protein were analyzed by densitometry (E).

Figure 6. *DEC1/Stra13* represses *PPARγ2* promoter activity

(A) NIH-3T3 cells were transiently co-transfected with the 0.6-kb *PPARγ2* promoter-driven luciferase construct (PPAR) or the promoter-less vector (pXP2) and *DEC1* fragments (0.3 µg DNA each) as indicated. Luciferase activities in cell lysates were measured 40-48 hr after transfection using a luminometer and presented as relative luminescence units ± s.d.

(B) Luciferase constructs (pXP2) with the 0.6-kb *PPARγ2* promoter (FL), *BsrGI-StuI* fragment alone (BrS), or deletion of *BsrGI-StuI* fragment (FLΔBrS) were co-transfected into NIH-3T3 cells with *DEC1* or its vector control at the ratio of 1:5 (*DEC1* or control to pXP2). In other experiments, *C/EBPα* or *C/EBPβ* were also co-transfected at the ratio of 1:1 (*C/EBP* to pXP2). A *Renilla* luciferase construct was also included to monitor transfection efficiency. Luciferase activities were measured as in (A).

(C) Schematic representation of the *PPARγ2* proximal promoter region. Putative *C/EBP* sites are displayed with the conserved bases capitalized.

Figure 7. Ectopic expression of *DEC1/Stra13* inhibits differentiation of 3T3-L1 preadipocytes

(A) L1 cells were retrovirally infected with HA-DEC1, DEC1, N1 or vector control (pLXSN) and then induced to differentiate as described. Oil Red O-stained cells were photographed (20x) on Day 5.

(B) L1 cells were retrovirally infected as in (A). Total RNA was isolated on Day 2 after IDM stimulation and was analyzed by Northern blotting as in Figure 3A.

(C) L1 cells were induced to differentiate with or without the TAT-N1 (DEC1 aa1-141) protein at indicated final concentrations. Cells were stained on Day 6 and photographed (20x).

DETAILED DESCRIPTION OF THE INVENTION

10 Hypoxia inhibits adipogenesis via HIF-1

To evaluate the effect of hypoxia on adipogenesis, L1 cells were treated at different O₂ tensions with the standard cocktail of adipogenic hormones, IDM (Figure 1A). Under normoxia (20% O₂), L1 cells differentiate into mature adipocytes loaded with fat droplets as indicated by Oil Red O staining. However, adipocyte differentiation is completely inhibited when L1 cells are induced to differentiate under hypoxia (0.01% or 2% O₂). No significant changes in cell death were detected in hypoxia-treated cells by the Trypan Blue exclusion assay when compared to the normoxic control. Hypoxic effects can be mimicked by iron-chelators (deferrioxamine or DFO), or divalent transition-metal ions (cobalt). Inhibition of L1 cell differentiation is observed when CoCl₂ or DFO is added to the treatment media throughout the course of induction (6 days). However, treatment with CoCl₂ or DFO for the first 2 days of induction with IDM is sufficient to prevent L1 cell differentiation (Figure 1B). Mouse embryonic fibroblast (MEF) cells were used to evaluate if the inhibition of adipogenesis by hypoxia was a general phenomenon. MEFs are induced to differentiate into adipocytes by IDM supplemented with 5 μ M rosiglitazone, a synthetic *PPAR* γ 2 ligand (Lehmann et al., 1995). Consistent with the literature, about 15-20% of the MEF cells differentiate into adipocytes (Alexander et al., 1998). Treatment of MEFs with CoCl₂ or DFO suppresses adipogenesis (Figure 1C). Similar observations are made when MEF cells are maintained under at 2% O₂.

To assess the role of HIF-1 in the inhibition of adipogenesis, MEFs in which each allele of *HIF-1 α* was flanked by *loxP* sites were used (Ryan et al., 2000). The *HIF-1 α* gene is efficiently excised when MEFs are transduced with adenovirus containing the cre recombinase gene (Seagroves et al., 2001). Both cre-treated and control MEF cells differentiate into adipocytes upon hormonal stimulation in the absence of hypoxia mimetics (Figure 2). When the *HIF-1 α* gene is deleted from the genome by cre, the *HIF-1 α* deficient MEF cells continue to undergo significant adipocyte differentiation in the presence of either CoCl₂ (up to 50 μ M) or DFO (up to 25 μ M) (Figure 2). In contrast, CoCl₂ or DFO significantly represses differentiation of the control-treated MEF cells under the same conditions (Figure 2). This result indicates that HIF-1 is involved in inhibition of adipogenesis by hypoxia.

Hypoxia inhibits induction of *PPAR γ 2* expression

The effect of hypoxia on the expression of three key transcription factors, *C/EBP β* , *C/EBP δ* and *PPAR γ* , during L1 differentiation was investigated (Rangwala and Lazar, 2000; Rosen and Spiegelman, 2000). At 20% O₂, both *C/EBP β* and *C/EBP δ* are significantly induced within 2 hr of IDM treatment, followed by their gradual decrease (Figure 3A and B). *PPAR γ 2* mRNA is induced by Day 2 post-IDM treatment following the expression of *C/EBP β* and *C/EBP δ* , and remains elevated throughout the rest of the differentiation process (Figure 3A and B). Under hypoxia, the induction of *PPAR γ 2* expression is completely abolished, and that of *C/EBP β* is reduced (Figure 3A and B). The *PPAR γ 2* expression is also repressed in L1 cells treated with CoCl₂ or DFO (Figure 3C). Unexpectedly, *C/EBP δ* expression becomes dysregulated and its mRNA remains elevated under hypoxia (Figure 3A and B). To assess the role of HIF-1 in *PPAR γ 2* inhibition, the *PPAR γ 2* mRNA in MEFs that were treated with cre to excise the *HIF-1 α* alleles were analyzed. As shown in Figure 3D, *PPAR γ 2* induction is protected in cre-treated MEFs, but not in mock-treated cells, indicating that HIF-1 is required for the inhibition of *PPAR γ 2* expression. These results suggest that negative regulation of *PPAR γ 2* and/or *C/EBP β* gene expression is a key mechanism for hypoxia-mediated inhibition of adipogenesis.

We next investigated whether overexpression of *C/EBP β* gene was sufficient to restore L1 cell differentiation under hypoxia. Consistent with the

literature (Yeh et al., 1995), *C/EBP β* -expressing L1 cells display much higher levels of differentiation than the vector-infected cells with or without stimulation by IDM under normoxic conditions (Figure 4A). At 2% O₂, the *C/EBP β* -expressing cells continue to differentiate into adipocytes, independent of IDM treatment (Figure 4A).
5 However, the differentiation of the vector-infected cells is completely inhibited at 2% O₂. When hypoxia mimetics are used, the continuous presence of CoCl₂ or DFO is necessary for significantly reducing differentiation and/or fat accumulation of the *C/EBP β* -expressing L1 cells (Figure 4B). If CoCl₂ or DFO is present for the first 2 days of adipogenic induction, the *C/EBP β* -expressing L1 cells are still able to
10 differentiate into fat-laden adipocytes (Figure 4B). Thus, while overexpression of *C/EBP β* can make L1 cells refractory to hypoxia or hypoxia mimetics, the level of adipocyte differentiation induced by IDM in these same cells is attenuated. This result suggests that hypoxia may be affecting additional modulators of adipogenesis induced by IDM.
15 We also determined whether overexpression of *PPAR γ 2* gene is sufficient to drive L1 differentiation under hypoxia. L1 cells received three rounds of *PPAR γ 2* retroviral gene infection to maximize infection efficiency. The *PPAR γ 2*-infected cells are induced to differentiate in medium containing 10% fetal bovine serum and 1 μ M rosiglitazone. Significant adipocyte differentiation is observed at
20 20% O₂ (Figure 4C), indicating efficient expression of *PPAR γ 2* gene. In contrast, vector-infected L1 cells do not differentiate under the same conditions although these cells can still differentiate upon IDM-stimulation. Similar to *C/EBP β* -transduced cells, the majority of the *PPAR γ 2*-expressing cells differentiate into adipocytes when stimulated by rosiglitazone under the conditions of 2% O₂, CoCl₂, or DFO but they
25 accumulate less fat as compared to normoxic controls (Figure 4C). Our results indicate that overexpression of *PPAR γ 2* or *C/EBP β* gene can overcome inhibition of adipocyte differentiation under hypoxia, but the phenotype of mature adipocytes is not fully restored.

***DEC1/Stra13* Is an Effector for Hypoxia-Mediated Inhibition of Adipogenesis**

30 Since HIF-1 is a transcription activator, the inhibition of adipogenesis by hypoxia is likely to be mediated by HIF-1 regulated genes. We investigated whether the HIF-1 target gene *DEC1/Stra13*, which contains a bHLH and an Orange

domain homologous to those of the HES transcription repressors (Boudjelal et al., 1997; Shen et al., 1997), is involved in inhibition of adipogenesis by hypoxia. HIF-1 is required for hypoxic induction of *DEC1/Stra13* since the increase of *DEC1/Stra13* mRNA occurs in wild-type MEF cells, but not in *HIF-1* $\alpha^{-/-}$ MEF cells (Figure 5A).

5 *DEC1/Stra13* protein is also sensitive to O₂ tensions as it increases rapidly under hypoxia and decreases to its basal level within 6-12 hr upon reoxygenation (Figure 5B). During L1 cell differentiation under normoxia, *DEC1/Stra13* mRNA is induced approximately 2 fold within 2 hr of IDM treatment (Figure 5C and E), which is consistent with the literature (Inuzuka et al., 1999). In contrast, *DEC1/Stra13* mRNA

10 in differentiating L1 cells exhibits approximately a 4-fold increase on Day 2, followed by a steady decline to the basal level under hypoxia (Figure 5C and E). Interestingly, the *DEC1/Stra13* protein level remains elevated (approximately 3 fold) from Day 2 through Day 8 under hypoxia as compared to normoxia (Figure 5D and E). The increased stability of *DEC1/Stra13* protein in IDM-stimulated L1 cells indicates that

15 both *DEC1/Stra13* mRNA and protein are regulated by hypoxia.

To address whether *DEC1/Stra13* could repress *PPAR γ 2* gene induction, the effect of DEC1 on the *PPAR γ 2* promoter activity was analyzed using the 0.6-kb *PPAR γ 2* promoter (-603 to +62)-driven luciferase gene as a reporter for *PPAR γ 2* transcriptional activity (Tong et al., 2000). As shown in Figure 6A, full-

20 length DEC1 represses *PPAR γ 2* promoter activity by 70% as compared to the vector control. Interestingly, two N-terminal fragments (N1 and N2) containing the bHLH domain show similar levels of repression as the full-length DEC1 (Figure 6A). In contrast, the two C-terminal fragments (C1 and C2) do not inhibit *PPAR γ 2* promoter activity. This result suggests that the bHLH domain of *DEC1/Stra13* is functionally

25 sufficient for inhibition of *PPAR γ 2* gene expression. Using a series of deletion or truncation constructs of the 0.6-kb *PPAR γ 2* promoter (FL), the *Bsr*GI (-285)-*Stu*I (-116) fragment (BrS) was found to contain the element(s) repressible by DEC1. Compared to the FL promoter, the BrS fragment shows similar levels of repression by DEC1, whereas the region with BrS deleted (FL Δ BrS) is no longer repressed by

30 DEC1 (Figure 6B). Structural analysis suggests a C/EBP β site at -229 (SEQ ID NO:5) and a C/EBP α site at -200 (SEQ ID NO:6) followed by a YY-1 box within BrS (Figure 6C). Co-transfection of C/EBP β or α can partially relieve repression by

DEC1 (Figure 6B). Further deletion of the C/EBP β site or both C/EBP sites from BrS results in 80% and 85% decrease in transcription activity alone, respectively. Together, these data suggest that DEC1 repression is mediated, at least in part, by the putative C/EBP sites at -229 and -200.

5 More importantly, overexpression of DEC1 or HA-DEC1 in L1 cells by retroviral infection results in nearly complete inhibition of adipocyte differentiation (Figure 7A). Significant inhibition is also conferred by retroviral transduction of N1 (amino acid aa1-141), as shown in Figure 7A. Inhibition of adipogenesis by DEC1 is consistent with the finding that *PPAR* γ 2 expression is
10 repressed in L1 cells ectopically expressing DEC1, HA-DEC1 or N1 (Figure 7B). As shown in Figure 6A, the bHLH domain of *DEC1/Stra13* is functionally equivalent to the full-length protein in repressing *PPAR* γ 2 expression. To more vigorously investigate the role of this bHLH domain in regulating adipogenesis, a fusion protein between N1 and an 11-aa protein transduction domain (YGRKKRRQRRR; SEQ ID
15 NO:7) derived from the TAT protein of human immunodeficiency virus (HIV) was constructed (Schwarze and Dowdy, 2000). A dose dependent inhibition of adipogenesis is found when L1 cells are induced to differentiate in the presence of the TAT-N1 fusion protein (Figure 7C). These results indicate that *DEC1/Stra13* functions as an effector of HIF-1 for the inhibition of adipogenesis by hypoxia.
20 Our results indicate that hypoxia-mediated adipogenic inhibition involves the repression of *PPAR* γ 2 gene induction and decrease in *C/EBP* β expression, two critical events during adipogenesis. The inhibition of *PPAR* γ 2 expression and its activity is a common mechanism for adipogenic inhibition by a variety of stimuli. A recent report has shown that HIF-1 can also repress *PPAR* α
25 gene expression (Naravula and Colgan, 2001). However, *PPAR* α may only play a minor role in white adipose tissue or preadipocytes as suggested by its low abundance (Braissant et al., 1996) and by gene knockout studies (Lee et al., 1995). The transcriptional activity of *PPAR* γ 2 protein is inhibited when phosphorylated by the mitogen-activated protein kinase (MAPK) (Hu et al., 1996). Expression of *PPAR* γ 2 is
30 repressed by the zinc-finger family transcription factors GATA-2 and GATA-3 that are expressed in preadipocytes but dissipate at the onset of adipogenic stimulation (Tong et al., 2000). Constitutive expression of *GATA*-2 or -3 prevents adipogenesis

by inhibiting *PPAR* γ 2 expression. Nevertheless, expression of *PPAR* γ 2 fails to completely rescue the adipogenic inhibition by *GATA*-2 or -3. Wnt-mediated signaling also inhibits the expression of *PPAR* γ 2 and *C/EBP* α and thereby represses the adipogenesis (Ross et al., 2000). Co-expression of *PPAR* γ 2 or *C/EBP* α with *Wnt*,
5 again, does not fully restore adipogenesis. Similar to these observations, expression of *PPAR* γ 2 can only partially restore the differentiation of L1 preadipocytes under hypoxia. These findings suggest that other *PPAR* γ 2-independent mechanisms also play important roles during adipocyte differentiation.

Retinoic acid (RA) inhibits the expression of *PPAR* γ and *C/EBP* α
10 without affecting *C/EBP* β expression (Schwarz et al., 1997). Overexpression of *C/EBP* α or *C/EBP* β does not overcome RA-mediated inhibition. It is interesting to note that *DEC1*/*Stra13* gene expression can also be induced by RA (Boudjelal et al., 1997), as well as by hypoxia, suggesting that *DEC1*/*Stra13* mediates repression of *PPAR* γ 2, but not *C/EBP* β . Thus, the similarity between RA- and hypoxia-mediated
15 adipogenic inhibition supports a role for *DEC1*/*Stra13* as a common effector of both RA- and hypoxia-induced inhibition of adipocyte differentiation.

DEC1/*Stra13* is well implicated in cell differentiation. *DEC1* was identified in differentiating human embryonic chondrocytes stimulated by a cyclic AMP analog (Shen et al., 1997). Its mouse homologue, *Stra13* (97% identical at
20 protein level), was identified during the RA-induced neuronal differentiation of P19 embryonal carcinoma cells (Boudjelal et al., 1997). In L1 cells, rapid but transient induction of *Stra13* mRNA was found upon IDM stimulation (Inuzuka et al., 1999), which may be important in temporally regulating transcription for adipogenesis. The regulation of the *PPAR* γ 2 promoter is still not understood and seems quite complex.
25 The data presented in the instant disclosure suggest that *DEC1*/*Stra13* represses the activation of *PPAR* γ 2 promoter potentially via the putative C/EBP sites at -229 and -200. However, unlike other members of the HES family, *DEC1*/*Stra13* does not bind to the E-box (CANNTG), the N-box (CACNAG), or the related C-box (CACGCG). It is unlikely that *DEC1*/*Stra13* binds any C/EBP site directly. Nevertheless, it is
30 possible that *DEC1*/*Stra13* may function through interaction with other transcription factors such as TBP, TFIIB, and USF (Boudjelal et al., 1997; Dhar and Taneja, 2001).

The exact nature of *DEC1/Stra13*-mediated inhibition of *PPAR γ 2* transcription warrants further investigation.

DEC1/Stra13 protein remains elevated in L1 cells under hypoxia even after its mRNA level decreases to the basal level. Such exceptional protein stability can potentially explain the incomplete recovery of adipogenesis under hypoxia even when *PPAR γ* or *C/EBP β* is overexpressed. The mechanism for increased protein stability of *DEC1/Stra13* warrants additional studies as it belongs to a small group of proteins including HIF-1 α and p53 that are regulated at the protein level under hypoxia (Graeber et al., 1994; Jewell et al., 2001). Preliminary examination suggests that each of the changes in stabilization of these proteins under hypoxia occurs by a different mechanism (Alarcon et al., 1999; Semenza, 1999; Z. Yun and A. J. Giaccia, unpublished observations).

In addition to the adipogenic hormones, the adipocyte microenvironment such as the extracellular matrix (Selvarajan et al., 2001) can also have significant influence on adipogenesis. The data presented in the instant disclosure strongly suggest that hypoxia, a physiological factor of the tissue microenvironment, may be an important regulator of adipogenesis. The importance of HIF-1 on adipocyte differentiation extends its role in regulating energy homeostasis. This mechanism of adipogenic repression may be potentially useful for controlling obesity by the regulation of HIF-1, *DEC1/Stra13* or pharmacological manipulation of intracellular O₂-sensing mechanisms.

In some embodiments of the invention, a mammalian cell or tumor may be contacted with an agent that directly or indirectly reduces the amount of active *PPAR γ 2* in a nucleus, a cell, and/or a tumor, which may result in inhibition of angiogenesis. Non-limiting examples of agents that may be used include *DEC1/Stra13* polypeptides (truncated or full-length), retinoic acid, antisense *PPAR γ 2*, anti-*PPAR γ 2* antibodies (mono- or polyclonal), and thiazolidinediones or combinations thereof. Artisans of ordinary skill may determine the amount of the agent necessary to result in inhibition of angiogenesis by either directly assaying the agent's effect on angiogenesis or by assaying the agent's effect on *PPAR γ 2* levels using any angiogenesis or *PPAR γ 2* assays known in the art. A non-limiting example of an angiogenesis assay is described in O'Reilly et al. 1994. Nonlimiting examples

of methods of assaying *PPAR* γ 2 levels include Mueller et al., 1998 and Palakurthi et al. 2001.

Agents of the invention may be administered for antiadipogenesis or antiangiogenesis by injection or gradual infusion subcutaneously, intraocularly, intravenously, intramuscularly, intracavity, orally, or transdermally. A therapeutically effective amount of an antiangiogenesis agent is the amount necessary to result in a measurable inhibition of either *de novo* angiogenesis or growth factor stimulated angiogenesis. A therapeutically effective amount of an antiadipogenesis agent is the amount necessary to result in a measurable inhibition of adipogenesis.

For thiazolidinediones, the concentration may be less than about 10 μ M (Berger et al. 2002) and may be up to about three orders of magnitude less than 10 μ M. A truncated *DEC1/Stra13* protein may be used at from about 0.2 μ M to about 5 μ M.

Agents of the invention may be used to inhibit angiogenesis in cells associated with any tumor. Nonlimiting examples of tumors that may be susceptible to antiangiogenic treatment include tumors of the colon, breast, and prostate. Nonlimiting examples further include tumors of the bladder, brain, cervix, connective tissue, endometrium, esophagus, liver, kidney, lung, lymph node, ovary, skin, intestine, stomach, testis, and uterus. Agents of the invention may also be used to for treatment of angiogenic dependent or angiogenic associated diseases, such as diabetic retinopathy, obesity, macula degeneration, rheumatoid arthritis, graft rejection such as corneal or kidney graft, angiomas, angiosarcomas, and Castelman disease and Kaposi sarcoma.

The invention provides methods of inhibiting angiogenesis in a tumor comprising contacting at least one cell of a tumor with a therapeutically effective amount of an agent of the invention selected from the group consisting of a *DEC1/Stra13* polypeptide (truncated or full-length), retinoic acid, an antisense *PPAR* γ 2 nucleic acid, an anti-*PPAR* γ 2 antibody (mono- or polyclonal), and a thiazolidinedione.

EXAMPLES

The examples provided in the instant disclosure are intended to further illustrate the invention and, therefore, shall not be construed to limit the scope of the instant invention.

5 **Example I: Reagents**

Cobalt Chloride (CoCl_2), deferoxamine mesylate (DFO), Oil Red O, insulin (INS), dexamethasone (DEX), and 3-isobutyl-1-methylxanthine (MIX) were purchased from Sigma (St. Louis, MO). Stock solution of rosiglitazone was prepared in dimethylsulfoxide (DMSO) from the Avandia™ tablets (GlaxoSmithKline
10 Pharmaceuticals).

Example II: Plasmids

The following constructs were made by PCR amplification of cDNA fragments using pGEM-DEC1 (T. Kawamoto, Hiroshima University, Japan) as template and in-frame cloning at the *EcoRI* into pcDNA3.1His or pLXSN: pLXSN-
15 DEC1, pLXSN-DEC1 aa1-141 (N1), pcDNA3.1-DEC1, pcDNA3.1-DEC1 aa1-141 (N1), pcDNA3.1-DEC1 aa1-203 (N2), pcDNA3.1-DEC1 aa121-412 (C1), and pcDNA3.1-DEC1 aa201-412 (C2). The TAT-N1 was constructed by PCR amplification of DEC1 aa1-141 and cloned in frame at the *EcoRI* into pTAT-HA (S. F. Dowdy, Washington University, St. Louis, MO). The pLXSN/HA-DEC1 was
20 made as follows. Full-length DEC1 was PCR-amplified with 5' *NheI* and 3' *XbaI*, and was cloned into pAS1 at the *NheI* in frame to the HA tag. The pAS1-DEC1 was cut with *NdeI*, filled-in by Klenow, and then cut with *EcoRI* to release the HA-tagged DEC1. Finally, the HA-tagged DEC1 was ligated into the pLXSN prepared by *XhoI* digest, Klenow fill-in, and then *EcoRI* digest. The following *PPAR* γ 2 promoter
25 constructs were made from pXP2-*PPAR* γ 2 (-603 to +62) (G. S. Hotamisligil, Harvard University, Boston, MA) by restriction digest, Klenow fill-in, and then ligation: *BsrGI* (-285)-*StuI* (-116) fragment (BrS) and deletion of *BsrGI*-*StuI* fragment (FLΔBrS). All constructs were verified by sequencing.

Example III: Cell Culture

30 NIH-3T3 (ATCC, Rockville, MD), *HIF-1* $\alpha^{-/-}$ mouse embryonic fibroblasts (MEFs) (Ryan et al., 1998), wild-type MEFs, and MEFs with *HIF-1* α

alleles flanked by *loxP* sites (Ryan et al., 2000) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin and streptomycin. 3T3-L1 preadipocytes (ATCC) were maintained in growth medium (GM) containing 10% bovine calf serum (ATCC) and 1 mM sodium pyruvate in DMEM.

Example IV: Adipocyte Differentiation and Oil Red O Staining

Based on previously described procedure (Wu et al., 1996), L1 cells were maintained in GM for 2 days after confluence. The confluent cells were treated (day 0) with differentiation medium (DM) containing 10% FBS, 10 µg/ml INS, 1 µM DEX and 0.5 mM MIX in DMEM for 2 days. Cells were then maintained in DMEM containing 10% FBS and 1 µg/ml INS, and the medium was replaced every other day.

MEFs were induced to differentiate 2 days after confluence (day 0) with DM supplemented with 5 µM rosiglitazone (Alexander et al., 1998; Lehmann et al., 1995). The medium was replaced on Day 3 with DMEM plus 10% FBS, 1 µg/ml INS and 5 µM rosiglitazone, and was then changed every other day. To remove the *HIF-1α* gene, subconfluent culture of MEFs with *HIF-1α* alleles flanked by *loxP* sites were treated with adenovirus containing cre recombinase or control adenovirus at the multiplicity of infection (MOI) of 50 as previously described (Seagroves et al., 2001). The treated MEF cells were grown to confluence for 2 days before they were induced to differentiate as above.

To evaluate the effects of hypoxia on adipogenesis, cells were maintained at 20% O₂ in standard incubator, 2% O₂ in standard incubator or 0.01% O₂ in an anaerobic chamber immediately following treatment with DM. Alternatively, CoCl₂ or DFO was added to DM at indicated concentrations either during the initial stage of stimulation or for the entire course of differentiation.

For visualization of differentiated adipocytes, cells were washed with phosphate-buffered saline (PBS) and stained in 60% of the Oil Red O stock solution (0.5 g Oil Red O in 100 ml of isopropanol) for 30 min at 37°C. Cells were briefly washed in 60% isopropanol and then rinsed in distilled water for microscopic observation and photography.

Example V: Retroviral Infection of 3T3-L1 Cells

Retroviruses were produced using the phoenix cell system (Baker et al., 1992; Pear et al., 1993). L1 cells at 30-50% confluence were retrovirally infected 2-3 times with 8 µg/ml Polybrene by centrifugation at 1800-2000 rpm for 1 hr, followed by overnight incubation at 32 °C. For generation of stable *C/EBPβ*-expressing cells, L1 cells were infected with pBABEpuro or pBABEpuro-*C/EBPβ* (S. R. Farmer, Boston University, Boston, MA), followed by selection with 1 µg/ml of puromycin (Sigma). The stable cells were grown to confluence and induced to differentiate as described above. For transient infection with pBABEpuro, pBABEpuro-*PPARγ2* (B. M. Spiegelman, Harvard University, Boston, MA), pLXSN/HA-DEC1, pLXSN-DEC1 and pLXSN-N1, L1 cells were spin-infected and induced to differentiate as described above.

Example VI: Northern and Western Blotting Analysis

Total cellular RNA was isolated with Trizol reagent (Life Technologies). The following plasmids were used for cDNA template preparations by restriction digest: MSV-*C/EBPβ* and MSV-*C/EBPδ* (S. L. McKnight, Univ. Texas Southwestern Medical Center, Dallas, TX), pSVsport-*PPARγ2* (B. M. Spiegelman), pBS-Stra13 (P. Chambon, INSERM, Strasbourg, France). Hybridization was carried out at 65°C for 6 to 12 hr. The radioactive blot was visualized on Storm 860 PhosphoImager (Molecular Dynamics, Sunnyvale, CA).

For Western blotting analysis, cell lysates were prepared on ice using 25 mM HEPES buffer, pH7.4, containing 1% NP-40, 150 mM NaCl, 2 mM EDTA, and a protease inhibitor cocktail (Complete™, Boehringer Mannheim). Equal amounts (25 µg/lane) of total cellular proteins were subjected Western blotting with polyclonal rabbit anti-Stra13 (P. Chambon) at 1:2000, followed by incubation with alkaline phosphatase-conjugated anti-rabbit IgG. Protein bands were visualized using ECF substrates (Amersham) on Storm 860 PhosphoImager.

Example VII: Preparation of TAT Fusion Protein

The TAT-N1 fusion protein was prepared from bacteria as described (Vocero-Akbani et al., 2001). The TAT-N1 protein was purified under native conditions using Ni-NTA metal affinity chromatography (Qiagen). After imidazole

elution, the TAT-N1 protein was desalted and kept at -80°C in phosphate-buffered saline containing 15% glycerol until use.

Example VIII: *PPAR* γ 2 Promoter Assay

The luciferase reporter construct (pXP2) under the control of the -603 to +62 *PPAR* γ 2 proximal promoter fragment (PPAR) or other deletional subclones was used as described (Tong et al., 2000). NIH-3T3 cells were transiently co-transfected with 0.3 μg each of pXP2 or PPAR and pcDNA3.1His construct expressing a DEC1 fragment using LipofectAmine Plus reagents (Life Technologies). Luciferase activities in cell lysates were measured in triplicates after 40-48 hr of incubation using Monolight 2010 Luminometer (Analytical Luminescence Laboratory, Ann Arbor, MI). The luciferase activity is expressed in relation to protein concentrations that vary little from well to well. Alternatively, a *Renilla* luciferase reporter was co-transfected as a control for transfection efficiency.

Example IX: Inhibition of Angiogenesis via *PPAR* γ 2

The efficacy of a DEC1 agonist may be assayed as follows. A truncated DEC1 polypeptide having the amino acid sequence of SEQ ID NO:2 may be delivered directly to a mammalian tumor cell in a mammal. DEC1 agonist delivery may consist of a single administration or multiple administrations over a period of time. Corresponding tumor cells to which no polypeptide is delivered (e.g. no injection or buffered saline injection only) may serve as negative controls. The invention contemplates further controls as desired or needed and will be apparent to one of ordinary skill in the art. An additional test group may consist of cells to which the DEC1 agonist is administered in combination with from about 0.1 μM to about 10 μM rosiglitazone. Each group may consist of from about 1 to about 10 or more mammals.

Angiogenesis is preferably monitored before the first administration and periodically thereafter. The effect of each treatment may be assessed by measuring endothelial cell proliferation in particular or more comprehensively as described in O'Reilly et al. 1994. *PPAR* γ 2 levels may be monitored according to either Mueller et al., 1998 or Palakurthi et al. 2001.

The results of these experiments, as contemplated by the present example, may show that the DEC1 agonist inhibits angiogenesis in a concentration-dependent manner and that the combined treatment with the DEC1 agonist and rosiglitazone results in a either a more complete inhibition of angiogenesis or inhibition at lower concentrations of one or both agents than when used alone. The effect of the combination therapy may be additive or more than additive.

Example X: Inhibition of Angiogenesis via *PPAR* γ 2

The efficiency of a DEC1 agonist may be assayed as follows. A viral or nonviral expression vector comprising a nucleic acid encoding a truncated DEC1 polypeptide having the amino acid sequence of SEQ ID NO:2 may be delivered to a mammalian tumor cell in a mammal. DEC1 agonist expression vector delivery may consist of a single administration or multiple administrations over a period of time. Corresponding tumor cells to which no vector is delivered (*e.g.* no injection or buffered saline injection only) may serve as negative controls. The invention contemplates further controls as desired or needed and will be apparent to one of ordinary skill in the art. An additional test group may consist of cells to which the DEC1 agonist expression vector is administered in combination with from about 0.1 μ M to about 10 μ M rosiglitazone. Rosiglitazone may be delivered in a single or multiple administrations. Each group may consist of from about 1 to about 10 or more mammals.

Angiogenesis is preferably monitored before the first administration and periodically thereafter. The effect of each treatment may be assessed by measuring endothelial cell proliferation in particular or more comprehensively as described in O'Reilly et al. 1994. *PPAR* γ 2 levels may be monitored according to either Mueller et al., 1998 or Palakurthi et al. 2001. In addition, expression of the DEC1 agonist by the expression vector may be assayed periodically by, for example, Northern or Western blotting techniques well known in the art. *See e.g.* Example VI.

The results of these experiments, as contemplated by the present example, may show that the DEC1 agonist inhibits angiogenesis in a concentration-dependent manner and that the combined treatment with the DEC1 agonist and rosiglitazone results in a either a more complete inhibition of angiogenesis or

inhibition at lower concentrations of one or both agents than when used alone. The effect of the combination therapy may be additive or more than additive.

REFERENCES

The references cited throughout this application and listed below are
5 incorporated herein in their entirety by reference. Citation of these documents is not to be construed as an admission that such documents are available as "prior art" against the instant invention.

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CLAIMS

We claim:

1. A truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR γ 2* promoter repressing activity as full-length *DEC1/Stra13* polypeptide.
2. The truncated polypeptide of claim 1, wherein the polypeptide contains the basic helix loop helix domain of *DEC1/Stra13*.
3. The truncated polypeptide of claim 1 having the amino acid sequence of amino acids 1-141 of SEQ ID NO:2.
4. The truncated polypeptide of claim 1 having the amino acid sequence of amino acids 1-141 of SEQ ID NO:4.
5. The truncated polypeptide of any one of claims 1, 2, 3, or 4 further comprising a peptide having the amino acid sequence of SEQ ID NO: 7.
6. A truncated *DEC1* polypeptide having substantially the same *PPAR γ 2* promoter repressing activity as a full-length *DEC1* polypeptide, wherein the truncated polypeptide consists essentially of a polypeptide having the amino acid sequence of amino acids 1-141 of SEQ ID NO:2.
7. A truncated *Stra13* polypeptide having substantially the same *PPAR γ 2* promoter repressing activity as a full-length *Stra13* polypeptide, wherein the truncated polypeptide consists essentially of a polypeptide having the amino acid sequence of amino acids 1-141 of SEQ ID NO:4.
8. An isolated nucleic acid encoding a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR γ 2* promoter repressing activity as full-length *DEC1/Stra13* polypeptide.
9. A method of inhibiting adipogenesis comprising:
contacting a cell with a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR γ 2* promoter repressing activity as full-length *DEC1/Stra13* polypeptide in an amount sufficient to repress *PPAR γ 2* promoter activity,

wherein expression of *PPAR* γ 2 is reduced and adipogenesis is inhibited.

10. The method of claim 9 wherein the truncated polypeptide contains the basic helix loop helix domain of *DEC1/Stra13*.
11. The method of claim 9 wherein the truncated polypeptide has the amino acid sequence of amino acids 1-141 of SEQ ID NO:2.
12. The method of claim 9 wherein the truncated polypeptide has the amino acid sequence of amino acids 1-141 of SEQ ID NO:4.
13. The truncated polypeptide of any one of claims 9, 10, 11, or 12 further comprising a peptide having the amino acid sequence of SEQ ID NO: 7.
- 10 14. A method of inhibiting *PPAR* γ 2 promoter activity comprising contacting a cell with a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR* γ 2 promoter repressing activity as full-length *DEC1/Stra13* polypeptide.
- 15 15. The method of claim 14 wherein the truncated polypeptide contains the basic helix loop helix domain of *DEC1/Stra13*.
16. The method of claim 14 wherein the truncated polypeptide has the amino acid sequence of amino acids 1-141 of SEQ ID NO:2.
17. The method of claim 14 wherein the truncated polypeptide has the amino acid sequence of amino acids 1-141 of SEQ ID NO:4.
- 20 18. The truncated polypeptide of any one of claims 14, 15, 16, or 17 further comprising a peptide having the amino acid sequence of SEQ ID NO: 7.
19. A method of inhibiting angiogenesis in a tumor comprising:
 - contacting the tumor with a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR* γ 2 promoter repressing activity as full-length *DEC1/Stra13* polypeptide in an amount sufficient to repress *PPAR* γ 2 promoter activity,
 - wherein expression of *PPAR* γ 2 is reduced and angiogenesis is inhibited.
20. The method of claim 19, wherein said truncated *DEC1/Stra13* polypeptide contains the basic helix loop helix domain of *DEC1/Stra13*.
21. The method of claim 19, wherein said truncated *DEC1/Stra13* polypeptide has the amino acid sequence of amino acids 1-141 of SEQ ID NO:2.

22. The method of claim 19, wherein said truncated *DEC1/Stra13* polypeptide has the amino acid sequence of amino acids 1-141 of SEQ ID NO:4.
23. The method of claim 19, wherein said tumor is selected from the group consisting of a colon tumor, a breast tumor, and a prostate tumor.
- 5 24. The method of claim 19, wherein said tumor is selected from the group consisting of tumors of the bladder, brain, cervix, connective tissue, endometrium, esophagus, liver, kidney, lung, lymph node, ovary, skin, intestine, stomach, testis, and uterus.
25. A method of inhibiting angiogenesis in an angiogenesis related disease comprising:
10 contacting at least one cell with a truncated *DEC1/Stra13* polypeptide lacking the *DEC1/Stra13* repressor domain wherein the truncated polypeptide has substantially the same *PPAR γ 2* promoter repressing activity as full-length *DEC1/Stra13* polypeptide in an amount
15 sufficient to repress *PPAR γ 2* promoter activity,
wherein expression of *PPAR γ 2* is reduced and angiogenesis is inhibited.
26. The method of claim 25, wherein said *DEC1/Stra13* polypeptide contains the basic loop helix domain of *DEC1/Stra13*.
27. The method of claim 25, wherein said truncated *DEC1/Stra13* polypeptide has
20 the amino acid sequence of amino acids 1-141 of SEQ ID NO:2 or SEQ ID NO: 4.
28. The method of claim 25, wherein said angiogenesis related disease is selected from the group consisting of diabetic retinopathy, obesity, macular degeneration, rheumatoid arthritis, graft rejection, angiosarcomas, Castleman
25 disease and Kaposi sarcoma.
29. A method of identifying a *DEC1/Stra13* agonist comprising:
 contacting a test compound with a cell comprising a reporter gene
 operably linked to a *PPAR γ 2* proximal promoter fragment; and
 comparing reporter gene expression in the presence of the test compound
30 with reporter gene expression in the presence of a truncated
 DEC1/Stra13 polypeptide lacking the *DEC1/Stra13* repressor domain
 wherein the truncated polypeptide has substantially the same *PPAR γ 2*
 promoter repressing activity as full-length *DEC1/Stra13* polypeptide,

wherein reporter gene expression that is about the same indicates a *DEC1/Stra13* agonists.

30. The method of claim 25 wherein the test compound is selected from the group consisting of an organic molecule less than about 3 kDa, a polypeptide, and a nucleic acid.
- 5
31. The method of claim 25 wherein the *PPAR* γ 2 proximal promoter fragment is the -603 to +62 *PPAR* γ 2 proximal promoter fragment.
32. A method of identifying a *PPAR* γ 2 agonist comprising:
- 10 contacting a test compound with a mammalian cell comprising a functional *PPAR* γ 2 gene; and
- comparing the amount of *PPAR* γ 2 polypeptide in the presence of the test compound with the amount of *PPAR* γ 2 polypeptide in the presence of a known *PPAR* γ 2 agonist,
- wherein reporter gene expression that is about the same indicates that the test compound is a *PPAR* γ 2 agonist.
- 15
33. The method of claim 28 wherein the test compound is selected from the group consisting of an organic molecule less than about 3 kDa, a polypeptide, and a nucleic acid.
- 20

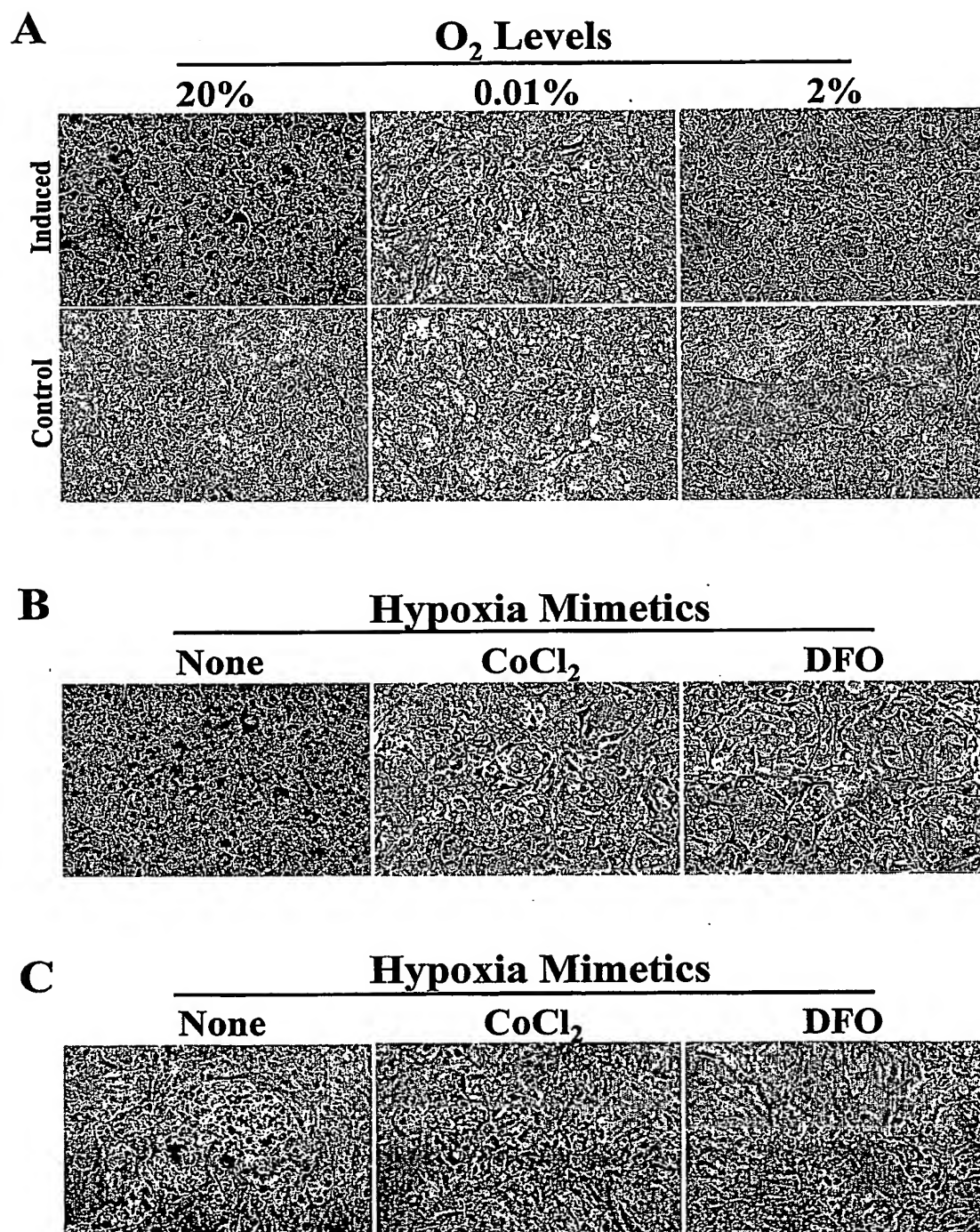
Figure 1

Figure 2

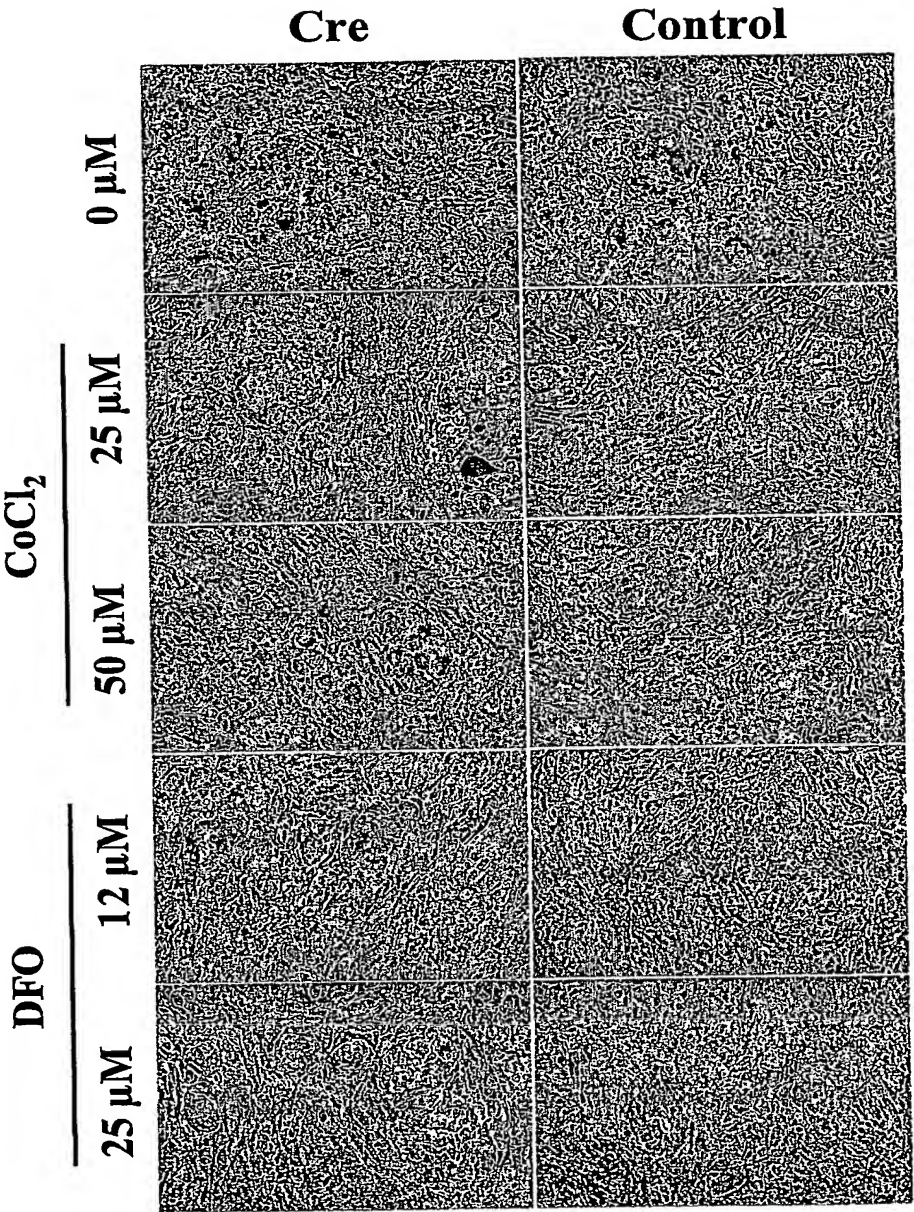


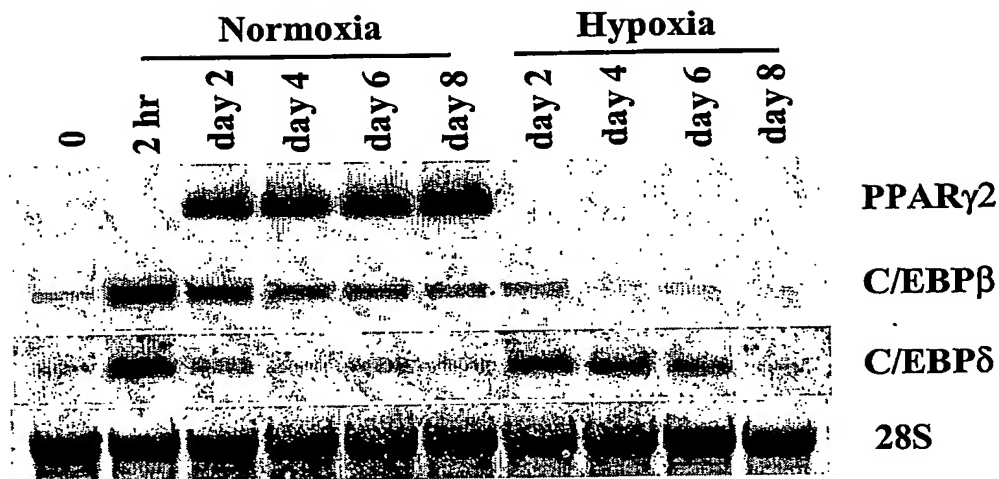
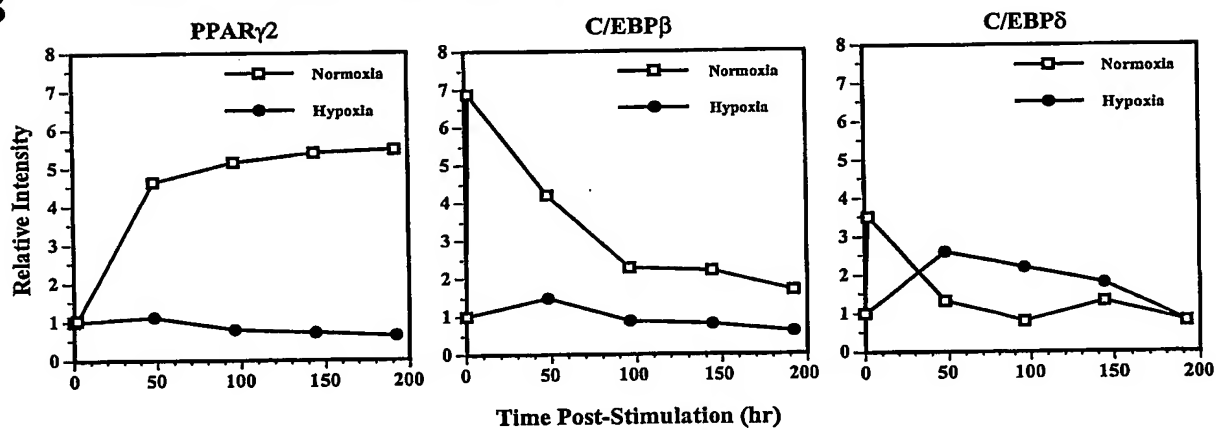
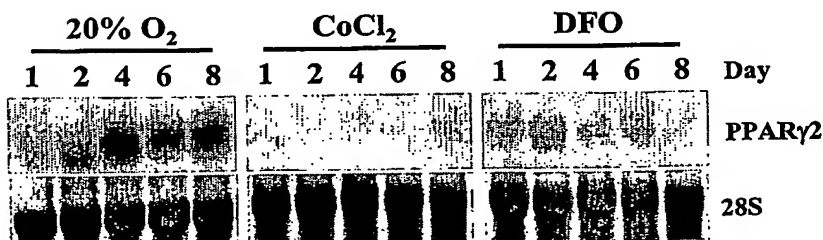
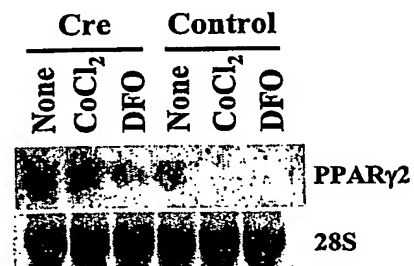
Figure 3**A****B****C****D**

Figure 4

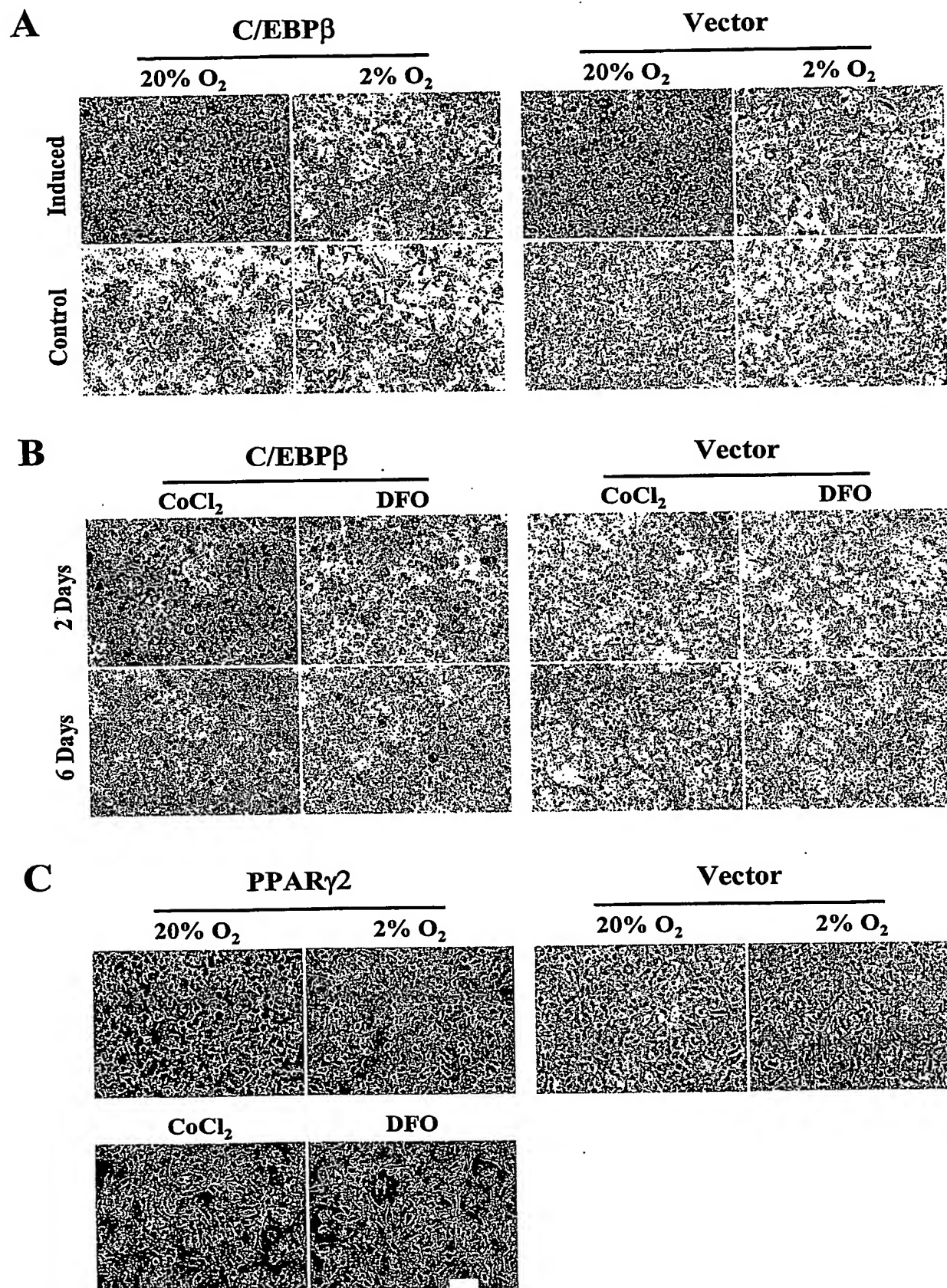


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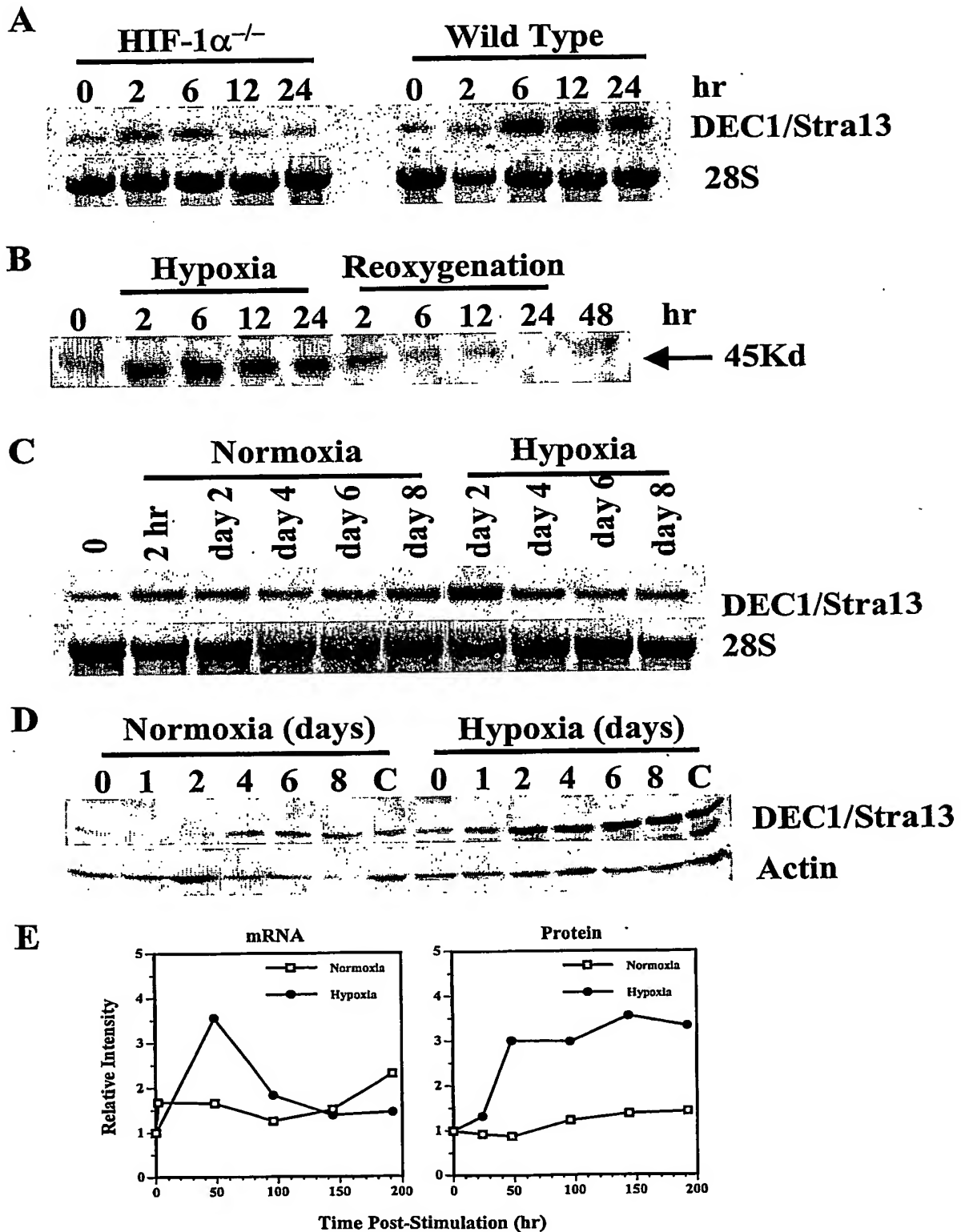
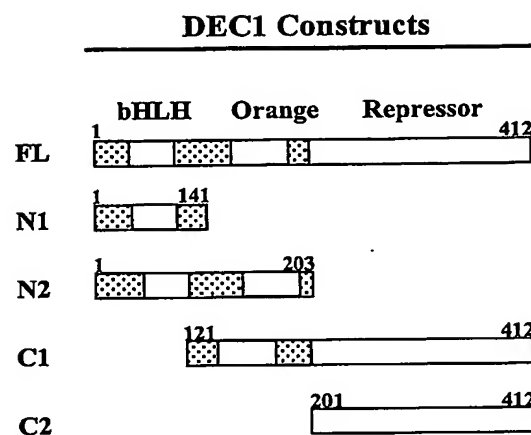
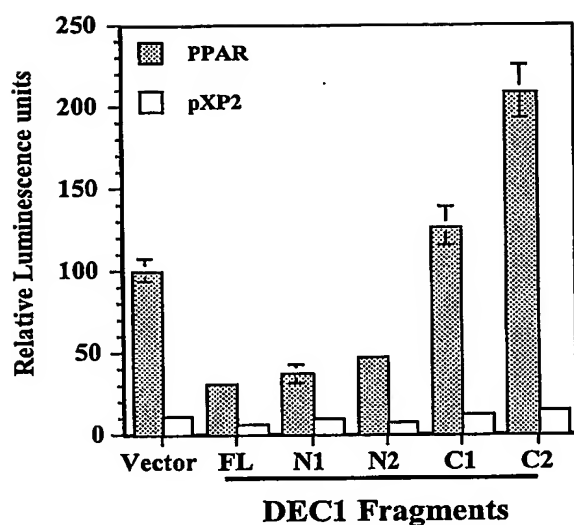
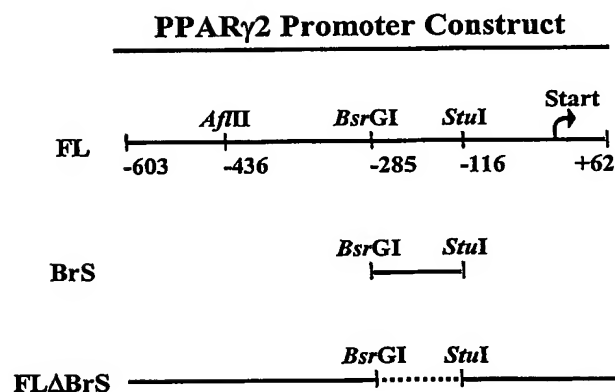
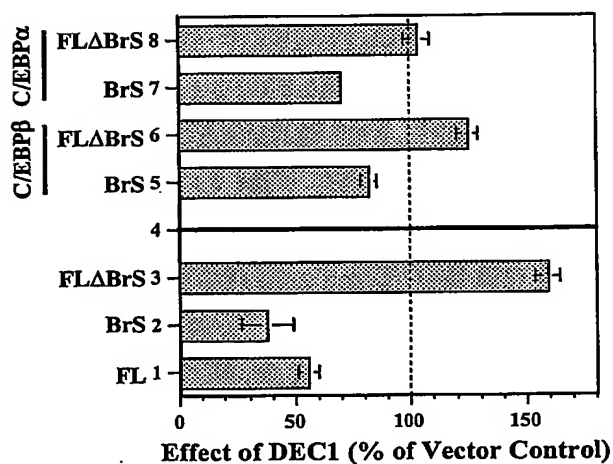


Figure 6

A



B



C

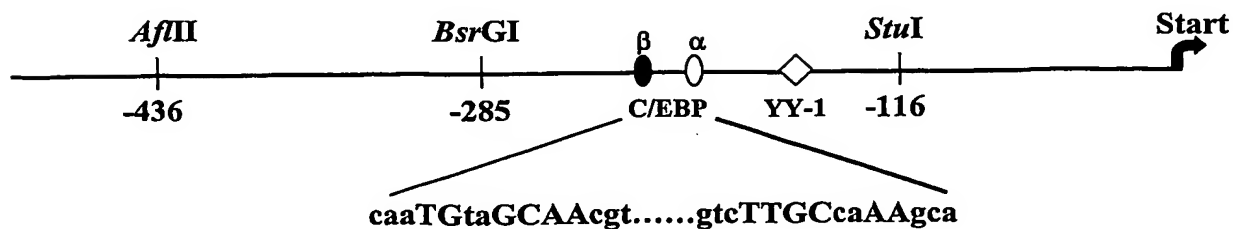
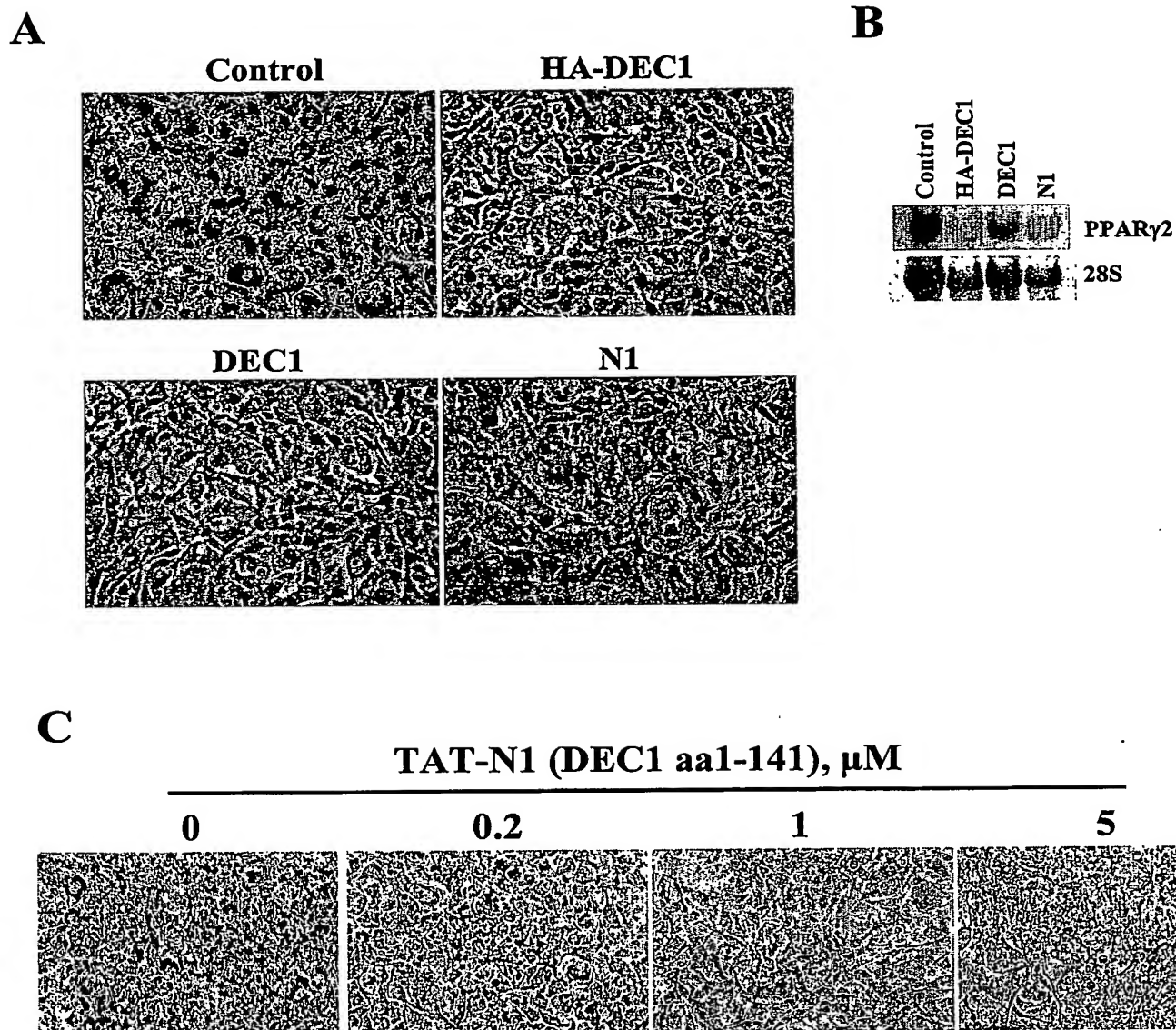


Figure 7



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<309> 2000-01-25

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WO 03/072064

PCT/US03/06360

Immunodeficiency Virus

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